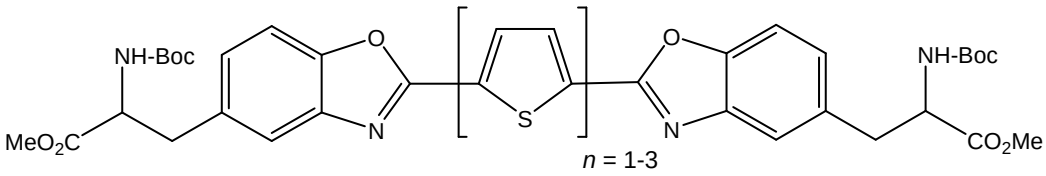
GRAPHICAL ABSTRACT

New highly fluorescent *bis*-amino acids based on alanine bearing (oligo)thiophene and benzoxazole units as the heterocyclic bridge were synthesized in moderate to good yields. Evaluation of the photophysical properties of the synthesized *bis*-amino acids revealed that they display exceptionally high fluorescence quantum yields, making them good candidates for application as fluorescent probes when incorporated into peptides, as well as peptide conformation-restricting and cross-linking elements.



Synthesis and photophysical characterization of new fluorescent *bis*-amino acids bearing a heterocyclic bridge containing benzoxazole and thiophene

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Abstract- A series of new *bis*-alanine derivatives bearing (oligo)thiophene and benzoxazole units as the heteroaromatic bridge were synthesized in moderate to good yields. The photophysical characterization of these *bis*-amino acids was performed by UV-visible absorption and fluorescence emission studies and revealed that the compounds displayed exceptionally high fluorescence quantum yields, making them good candidates for application as fluorescent probes when incorporated into peptides, as well as peptide conformation-restricting and cross-linking elements.

Keywords*: Bis*-amino acids; (Oligo)thiophene; Benzoxazole; Fluorescence; Fluorescent probes; *Bis*-alanines.

1. Introduction

Bis-amino acids have been the subject of significant research in recent years because they can be used in the synthesis of analogues of biologically active peptides[1] and also in biomimetic chemistry as cross-linking elements for the control of peptide secondary structure, leading to stabilized α-helical, β-turn and γ-turn conformations.[2] *Bis*-amino acids can be regarded as a replacement for cysteine residues in the cross-linking between peptide chains, and other residues like lysine, tyrosine and aspartic and glutamic acids which can also undergo cross-linking in the presence of appropriate reagents. Cross-linking is a key feature in (bio)polymer and biomaterials sciences and food chemistry.[3]

The insertion of noncoded amino acids into the backbone of both natural and synthetic polymers is a very appealing area of research since it can lead to the development of macromolecules possessing biomimetic characteristics, with unique structural and biological

properties. By synthetic manipulation at the side chain of coded amino acids, new functions and functional relationships can be generated as well as altered physicochemical properties, such as luminescence, conducting ability and metal ion and other analyte recognition ability, among other properties.[4]

The molecular design of functional peptides can thus be achieved by judicious choice of unnatural amino acids of natural and synthetic origin which are effective building blocks for peptide design considering the following two points: *i)* use of rigid unnatural amino acids to control the conformation and function of the resulting peptides and *ii)* incorporation of functional unnatural amino acids into peptides resulting in the appearance of the inherent functions.[5]

Benzoxazoles display excellent optical properties (broad spectral windows, high molar absorptivity coefficients and fluorescence quantum yields) and they have been described as fluorescent probes and sensing materials, namely as fluorescent and/or colorimetric sensors for metals, anionic species and in pharmaceutical analysis.[6] Thiophene and its derivatives also possess important electroluminescent properties with wide application in polymer science, which has prompted their application as energy transfer and light-harvesting systems, for optical and electronic devices,[7] as sensors and as fluorescent markers.[8]

The work on *bis*-amino acids reported so far deals with the synthesis of aliphatic- and aromatic-bridged *bis*-amino acids and, to the best of our knowledge, this is the first report on the synthesis of heteroaromatic-bridged *bis*-amino acids, apart from those concerning histidinoalanine.[9] Therefore, there is a practical interest on the synthesis of new and more complex systems and in order to contribute to and expand the body of work published in this area in the last years, we decided to design new *bis*-amino acid-based systems consisting on functionalized alanines containing thiophene and benzoxazole as the heterocyclic bridge, as a result of the previously mentioned applications for these heterocycles. These *bis*-alanine derivatives represent both a conformation-restricting and a functional amino acid, in view of their fluorescence. The resulting *bis*-(oligo)thienyl-benzoxazole-alanine derivatives, because of the presence of amino and carboxyl groups, could be incorporated into peptide chains and as such used as an energy donor in conformational studies of peptides by means of fluorescence or be used as fluorescence markers. Following our previous research on the synthesis and characterization of unnatural amino acids,[10] benz-X-azole derivatives with interesting optical properties[11] and heterocyclic colorimetric/fluorimetric chemosensors containing (oligo)thiophene, benzoxazole and amino acid moieties,[12] we now report the synthesis of a new highly fluorescent family of alanine derived *bis*-amino acids connected

through an (oligo)thienylbenzoxazole bridge, also having in mind the future studies of these compounds as fluorimetric peptide-based chemosensors. Amino acids and peptides are known to bind a variety of metal ions as they contain a large number of potential donor atoms through the peptide backbone and amino acid side chains and the insertion of an extended heterocyclic system between the two ends of the *bis*-amino acid will provide an UV-active and highly fluorescent chromophore, as well as additional binding sites through the donor atoms in the conjugated π-bridge. With the study of these new *bis*-(oligo)thienyl-benzoxazolyl-alanines we also intended to evaluate the effect of the length of the π-conjugated (oligo)thienyl system at position 2 of the benzoxazole moiety in the photophysical properties of the resulting compounds.

2. Results and discussion

2.1. Synthesis

The new *bis*-(oligo)thienyl-benzoxazolyl-alanines **4a-d** with thiophene, bithiophene, terthiophene and phenylthiophene units acting as linking π-conjugated bridges between the two benzoxazolyl-alanine systems were synthesized in good yields, by a multistep synthesis. Different (oligo)thiophenes were used in order to study the influence of the structure modification (*i.e.* the increase of the π-conjugated bridge) on the overall optical properties of compounds **4a-d** and also in order to compare with our recently reported systems containing only one benzoxazolyl-alanine system.[10c,12b]

2.1.1. Synthesis of diformyl-terthiophene 2c

The diformyl-terthiophene **2c** was synthesized through a Suzuki cross-coupling reaction of 5'-bromo-5-formyl-2,2'-bithiophene **1** with 5-formylthiophene boronic acid in 75% yield. Precursor **1** was obtained from commercial 5-formyl-2,2'-bithiophene by bromination with NBS in DMF in 98 % yield (Scheme 1).

< SCHEME 1 >

The synthesis of compound **2c** has been reported earlier by Giannetas et al[13] through Vilsmeier formylation of terthiophene. This reaction gave a mixture of three products which were isolated by column chromatography: the starting material, the mono-formyl derivative (65%) and the diformyl-terthiophene **2c** in 20% yield.

In comparison to the method described earlier, compound **2c** was obtained by us in higher yield from low cost commercially available reagents and using simple work-up procedures, allowing the good yielding preparation and ease of isolation of this derivative. Attempt to synthesize compound **2c** using the same method described by Giannetas, gave, in our hands, a complex mixture of compounds which was not possible to separate neither by recrystallization nor by chromatography.

2.1.2. Synthesis of *bis*-[2-(oligothienyl)benzoxazol-5-yl]-alanine derivatives 4a-d

Starting from commercially available 3-nitro-L-tyrosine, *N*-Boc-3-amino-L-tyrosine methyl ester **3** was obtained by using simple synthetic procedures.[12b] Condensation of compound **3** with diformylated (oligo)thiophenes **2a-d**, namely thiophene, bithiophene, terthiophene and phenylthiophene, resulted in the corresponding imines, which by subsequent oxidation and cyclisation with lead tetraacetate in DMSO afforded the *bis*-[2-(oligothienyl)benzoxazol-5-yl]-alanine derivatives **4a-d** in moderate to good yields (Scheme 2, Table 1). These compounds were fully characterised by the usual spectroscopic techniques.

< SCHEME 2 >

< TABLE 1>

The IR spectra of bis-amino acids **4a-d** showed the characteristic bands due to stretching vibrations of the carbonyl of the *C*- and *N*-terminal protecting groups: the methyl ester from 1744 to 1749 cm^{-1} and the *N*-benzyloxycarbonyl from 1693 to 1715 cm^{-1}. A band related to the oxazole ring (N=C stretching) appeared at 1633 to 1668 cm^{-1}.

^{1}H NMR spectra showed signals that were attributed to the benzoxazole, in the form of doublets at about δ 7.48 and 7.53 ppm for H7 and H4, respectively, and double doublets for H6 around δ 7.15 ppm. The confirmation of the ocurrence of the oxidative cyclization was also supported by ^{13}C NMR spectra, where signals of the oxazole C2 carbon were visible at about δ 159 ppm.

2.2. Photophysical study

The absorption and emission spectra of *bis*-(oligo)thienylbenzoxazolyl-alanines **4a-d** were measured in absolute ethanol (10^{-6}-10^{-5} M solution) (Table 1). The nature of the thiophenic bridge at position 2 of the benzoxazole had a clear influence on the absorption and emission bands of compounds **4a-d**. Comparing the absorption data for compounds **4b** and **4d**, it can be

seen that the replacement of the second thiophene ring by a phenyl ring leads to a small hypsochromic shift, related to the bathocromic effect of sulfur and an increased π-overlap between the thiophene units in compound **4b**. Also, compounds **4a**-**d** possess very large molar absorptivity coefficients (higher than 46,000 mol^{-1} L cm^{-1}).

The wavelength of maximum absorption for compounds **4a**-**c** was shifted to longer wavelengths as the number of thiophene units increased**,** as expected from the increase in conjugation. The same trend was observed in the emission spectra of these compounds as the position of the wavelength of maximum emission was red-shifted, *ca*. 40 nm for each added thiophene (Figure 1). The synthesized compounds showed large Stokes' shift (the lowest being 3698 cm^{-1} for **4a** and the highest 4821 cm^{-1} for **4d**). As Stokes' shifts directly relate to energy differences, a increasing trend could be observed along the series **4a**-**d**.A large Stokes' shift is an interesting characteristic for a fluorescent probe that allows an improved separation of the light inherent to the matrix and the light dispersed by the sample.[14]

< FIGURE 1 >

The relative fluorescence quantum yields were determined using a 10^{-6} M solution of 9,10-diphenylanthracene in ethanol as standard (Φ_F = 0.95)[15] and *bis*-(oligo)thienylbenzoxazolyl-alanines **4a-d** exhibited good to excellent fluorescence quantum yields. For the Φ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for each one of the compounds to be tested and in all fluorimetric measurements the absorbance of the solution did not exceed 0.1. The highest values were obtained for *bis*-alanines **4a**, **4b** and **4d**, with a thiophene, bithiophene and a phenylthiophene at position 2 of the benzoxazole, respectively, which were found to be strongly emissive ($0.59 < \Phi_F < 0.89$) while compound **4c**, with a terthiophene bridge, displayed much lower quantum yield ($\Phi_F = 0.14$).

At this stage, a comparison could be made between the photophysical data of the synthesized *bis*-alanines **4a-c** and the corresponding (oligo)thienylbenzoxazolyl-alanines **5a-c**, recently reported by us,[10c,12b] bearing only one benzoxazolyl-alanine system (Figure 2, Table 1). For all compounds, it was found that the introduction of a second benzoxazolyl-alanine system resulted in a bathochromic shift of both the absorption and emission wavelength maxima, an increase in the the molar absorptivity value and, more importantly, in an enhancement of the fluorescence quantum yield. Also, the new *bis*-alanines **4a**-**c** had smaller Stokes' shifts than the mono-alanine series **5a-c**, a fact that can potentially be rationalized by the more polar

nature of the mono-alanine compounds when compared to the inherently less polar nature of the *bis*-alanine compounds.

< FIGURE 2 >

Our results revealed a decrease in the fluorescence quantum yield as the number of thiophene rings at the π-conjugated bridge increased. Nevertheless, the fluorescence quantum yield of non substituted (oligo)thiophenes is expected to increase as the oligothiophene chain length increases, due to a further extension of the conjugated π-system. On the other hand, the heavy atom induced spin-orbit coupling by the sulphur atoms can give raise to a very efficient intersystem crossing mechanism, thus lowering the emission.[16] Moreover, azomethine nitrogens contribute to the heavy atom effect concomitant with the increased degree of conjugation.[16c] Also, the different chains (thiophene, bithiophene and terthiophene) should exihibt different degrees of torsion between the thiophene units, which leads to variations in the effective conjugation length, affecting the planarity of the whole heteroaromatic system.[16d] In our case, we believe that a combination of the above mentioned effects could be responsible for the trend observed in our results.

Keeping in mind further applications of these amino acids as emissive probes for energy transfer or FRET (Fluorescence Resonance Energy Transfer) studies when incorporated in peptides chains, in Figure 1 can also be seen the good superposition of some absorption and emission spectra (for example compounds **4c** and **4a)**, which opens up a very wide range of potential interesting applications to be explored.

3. Conclusions

In summary, we have achieved for the first time the synthesis of new fluorescent heteroaromatic *bis*-amino acid derivatives **4a-d** containing (oligo)thiophene and benzoxazole moieties combined with an alanine residue by simple procedures in good yields and their photophysical properties were evaluated. Due to their interesting photophysical properties, namely their strongly emissive character, these heterocyclic alanine derivatives could find application as useful building blocks for the cross-linking of peptide chains with the extra value of inserting a UV-active and fluorescent chromophore, adding functionality to the

resulting peptide. These heteroaromatic *bis*-amino acids could also be used as fluorescent markers and probes for FRET studies in peptides.

4. Experimental

4.1. Synthesis general

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230-240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer using KBr discs or nujol. UV-visible absorption spectra (200 – 800 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. ^{1}H NMR spectra were recorded on a Varian 300 spectrometer in $CDCl_3$ at 300 MHz at 25 ºC. All chemical shifts are given in ppm using δ_H Me_4Si = 0 ppm as reference and *J* values are given in Hz. ^{13}C NMR spectra were run in the same instrument at 75.4 MHz using the solvent peak as internal reference. Assignments were made by comparison of chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear HMBC and HMQC correlation techniques. Mass spectra were obtained on a Finnigan LXQ MS spectrometer (ESI technique, positive mode). Elemental analyses were carried out on a Leco CHNS 932 instrument. Fluorescence spectra were collected using a Spex Fluorolog 1680 Spectrometer. All reagents were used as received. 5-Formyl-2,2'-bithiophene, 3-nitro-L-tyrosine and 2,5-diformylthiophene **2a** are commercially available (Aldrich). The synthesis of 5,5'-diformyl-2,2'-bithiophene **2b**[11g] and 2-formyl-5-(4'-formylphenyl)-thiophene **2d** was described elsewhere.[11c]

4.2. Procedure for the synthesis of diformyl-terthiophene 2c

4.2.1. Synthesis of 5'-bromo-5-formyl-2,2'-bithiophene 1

To a stirred solution of 5-formyl-2,2'-bithiophene (0.8 g, 4.1 mmol) in DMF (1 mL), in the dark, was added NBS (0.73 g, 4.1 mmol) at -20 ^{o}C during 4 h. After this time the reaction mixture was poured on ice and stirred till room temperature and a light yellow solid precipitated, which was isolated by filtration. Recrystallization from dichloromethane-light petroleum gave the pure compound as a light yellow solid (1.09 g, 98 %). Mp = 137 – 139 ºC. IR (KBr): ν = 1651 (C=O), 1510, 1455, 1278, 1230, 1193, 1050, 975, 879, 800, 784, 751, 666, 644 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 7.05 (d, 1H, *J*= 3.9 Hz, H-3'), 7.12 (d, 1H, *J*= 3.9 Hz,

H-4'), 7.19 (d, 1H, *J*= 3.9 Hz, H-3), 7.67 (d, 1H, *J*= 3.9 Hz, H-4), 9.88 (s, 1H, C*H*O) ppm. $C_9H_5BrOS_2$: calcd. C 39.57, H 1.84, S 23.48; found C 39.65, H 1.75, S 23.42.

4.2.2 Synthesis of 5,5''-diformyl-2,2':5'2''-terthiophene 2c through Suzuki cross-coupling

5'-Bromo-5-formyl-2,2'-bithiophene **1** (0.60 g, 2.1 mmol) was coupled to 5-formylthiophene boronic acid (0.42 g, 2.7 mmol), in a mixture of DME (35 mL) and aqueous 2 M Na_2CO_3 (2 mL) and $Pd(PPh_3)_4$ (6 mol %) at 80 ºC under a argon atmosphere during 20 h. After cooling the mixture was filtered. Ethyl acetate and a saturated solution of NaCl were added and the phases were separated. The organic phase was washed with water (3 × 75 mL) and a aqueous solution of NaOH (10%). The organic phase obtained was dried ($MgSO_4$), filtered and solvent removal gave the crude product which was recrystallized affording the pure diformyl derivative **2** as an orange solid (0.38 g, 60 %). Mp = 203.6 – 205.2 ºC (lit.[13] 224 ºC). IR (nujol): ν = 1664 (C=O), 1505, 1319, 1219, 1164, 1047, 798, 759, 668 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 7.28-7.38 (m, 4H, H-3, H-3', H-3'' and H-4''), 7.70 (d, 2H, *J* = 4.2 Hz, H-4 and H-4''), 9.89 (s, 2H, 2 × C*H*O) ppm.

4.3. General procedure for the synthesis of *bis*-(oligo)thienylbenzoxazolyl-alanines 4a-d

N-*t*-Butyloxycarbonyl-3-amino-L-tyrosine methyl ester **3** (2 equiv.) was stirred with the corresponding diformylated (oligo)thiophene **2** (1 equiv.) and heated in ethanol overnigth at reflux (5 mL/equiv). The solvent was evaporated and the crude imine used in the next step without further purification. The corresponding crude imine and lead tetraacetate (3 equiv.) were stirred at room temperature in DMSO (5 mL/equiv.) for 3 days. The mixture was poured over water and extracted with ethyl acetate (3 × 10 mL). After drying with anhydrous magnesium sulphate and evaporation of the solvent under reduced pressure, the crude compound was submitted to column chromatography with silica gel by elution with dichloromethane and dichloromethane/methanol, 100:1. The fractions containing the pure compound were combined and evaporated under reduced pressure.

4.3.1. *bis*-[*N*-*t*-Butyloxycarbonyl [2-(thien-2'-yl)benzoxazol-5-yl]-L-alanine methyl ester] (4a). The product was isolated as a yellow solid (0.19 g, 75%). Mp = 106.2-108.0 ºC. IR: ν = 3355, 2968, 2940, 1749, 1693, 1640, 1583, 1524, 1476, 1446, 1392, 1365, 1255, 1166, 1056, 1016, 813, 794, 714 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.43 (s, 18H, 2 × $C(CH_3)_3$), 3.16-3.31 (m, 4H, 2 × β CH_2), 3.75 (s, 6H, 2 × CH_3), 4.62-4.67 (m, 2H, 2 × αH), 5.06 (d, *J* = 7.8 Hz, 2H, 2 ×

NH), 7.16 (dd, *J* = 1.8 and 8.4 Hz, 2H, 2 × H-6), 7.50 (d, *J* = 8.4 Hz, 2H, 2 × H-7), 7.53 (d, *J* = 1.8 Hz, 2H, 2 × H-4), 7.93 (dd, *J* = 1.2 and 3.9 Hz, 2H, 2 × H-3') ppm. ^{13}C NMR ($CDCl_3$): δ = 28.20 (C($\underline{C}H_3)_3$), 38.28 (β CH_2), 52.31 (CH_3), 54.53 (αC), 79.95 ($\underline{C}(CH_3)_3$), 110.25 (C7), 120.30 (C4), 126.18 (C6), 130.02 (C2'), 130.17 (C3'), 132.83 (C5), 142.27 (C3a), 149.61 (C7a), 155.14 (C=O Boc), 159.50 (C2), 171.97 (C=O ester) ppm. UV/Vis (ethanol, nm): λ_{max} (ε) = 371 (47,210). MS: *m/z* (ESI, %): 721 ([M+H]$^+$, 31), 665 (100), 609 (32). $C_{36}H_{40}N_4O_{10}S$: calcd. C 59.99, H 5.59, N 7.77; found C 60.04, H 5.67, N 7.61.

4.3.2. *bis*-[*N*-*t*-Butyloxycarbonyl[2-(bithien-5'-yl)benzoxazol-5-yl]-L-alanine methyl ester] (4b). The product was isolated as a orange solid (0.09 g, 54%). Mp = 111.7-113.9 ºC. IR (KBr): ν = 3415, 2965, 2932, 1745, 1714, 1633, 1579, 1473, 1394, 1369, 1260, 1164, 1053, 1019, 936, 863, 801, 713 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.43 (s, 18H, 2 × C($CH_3)_3$), 3.18-3.28 (m, 4H, 2 × β CH_2), 3.75 (s, 6H, 2 × CH_3), 4.60-4.65 (m, 2H, 2 × α-H), 5.03 (d, *J* = 8.1 Hz, 2H, 2 × NH), 7.15 (dd, *J* = 1.2 and 8.4 Hz, 2H, 2 × H-6), 7.36 (d, *J* = 4.2 Hz, 2H, 2 × H-4'), 7.48 (d, *J* = 8.4 Hz, 2H, 2 × H-7), 7.51 (d, *J* = 1.2 Hz, 2H, 2 × H-4), 7.85 (d, *J* = 4.2 Hz, 2H, 2 × H-3') ppm. ^{13}C NMR ($CDCl_3$): δ = 28.22 (C($\underline{C}H_3)_3$), 38.25 (β CH_2), 52.32 (CH_3), 54.60 (αC), 80.04 ($\underline{C}(CH_3)_3$), 110.22 (C7), 120.23 (C4), 126.45 (C4'), 126.28 (C6), 127.38 (C2'), 130.91 (C3'), 132.85 (C5), 143.84 (C5'), 142.37 (C3a), 149.65 (C7a), 155.09 (C=O Boc), 159.01 (C2), 172.10 (C=O ester) ppm. UV/Vis (ethanol, nm): λ_{max} (ε) = 388 (46,641). MS: *m/z* (ESI, %): 803 ([M+H]$^+$, 34), 747 (30), 681 (78), 509 (30), 451 (100). $C_{40}H_{42}N_4O_{10}S_2$: calcd. C 59.84, H 5.27, N 6.98; found C 59.78, H 5.27, N 7.11.

4.3.3. *bis*-[*N*-*t*-Butyloxycarbonyl[2-(terthien-5'-yl)benzoxazol-5-yl]-L-alanine methyl ester] (4c). The compound was isolated as a orange solid (0.07 g, 60 %). Mp = 99.1-101.3 ºC. IR (KBr): ν = 3428, 2976, 2929, 1744, 1714, 1662, 1577, 1516, 1496, 1439, 1392, 1366, 1256, 1218, 1165, 1060, 1018, 913, 863, 792 cm^{-1}. ^{1}H RMN ($CDCl_3$): δ = 1.44 (s, 18H, 2 × C($CH_3)_3$), 3.15-3.24 (m, 4H, 2 × β CH_2), 3.75 (s, 6H, 2 × CH_3), 4.60-4.66 (m, 2H, 2 × α-H), 5.04 (d, *J* = 8.1 Hz, 2H, 2 × NH), 7.14 (dd, *J* = 1.8 and 8.1 Hz, 2H, 2 × H-6), 7.34 (d, *J* = 3.9 Hz, 2H, 2 × H-4'), 7.48 (d, *J* = 8.1 Hz, 2H, 2 × H-7), 7.51 (d, *J* = 1.8 Hz, 2H, 2 × H-4), 7.71 (d, *J* = 3.9 Hz, 2H, 2 × H-3'), 7.86 (s, 2H, 2 × H-3'') ppm. ^{13}C NMR ($CDCl_3$): δ = 28.23 (C($\underline{C}H_3)_3$), 38.17 (β CH_2), 52.24 (CH_3), 54.60 (α C), 80.03 ($\underline{C}(CH_3)_3$), 110.17 (C7), 120.16 (C4), 124.30 (C4'), 125.74 (C3''), 126.30 (C6), 127.26 (C2'), 130.81 (C3'), 132.78 (C5), 137.83 (C2''), 141.93 (C5'), 142.28 (C3a), 149.67 (C7a), 155.11 (C=O Boc), 158.91 (C2), 172.12 (C=O ester) ppm. UV/Vis (ethanol, nm): λ_{max} (ε) = 416 (46,454 MS: *m/z* (ESI, %):

885 ([M+H]$^+$, 22), 509 (100). $C_{44}H_{44}N_4O_{10}S_3$: calcd. C 61.97, H 5.20, N 6.57; found C 61.88, H 5.27, N 6.72.

4.3.4. *bis*-[*N*-*t*-Butyloxycarbonyl[2-(phen-4''-yl-thien-2'-yl)benzoxazol-5-yl]-L-alanine methyl ester] (4d). The compound was isolated as a yellow solid (0.14 g, 76 %). Mp = 96.00-98.5 ºC. IR (KBr): ν = 3435, 2977, 2930, 1746, 1715, 1668, 1615, 1575, 1505, 1479, 1367, 1257, 1168, 1056, 1018, 802 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.44 (s, 18H, 2 × $C(CH_3)_3$), 3.20-3.25 (m, 4H, 2 × β CH_2), 3.75 (s, 6H, 2 × CH_3), 4.62-4.66 (m, 2H, 2 × α-H), 5.05 (d, *J* = 8.4 Hz, 2H, 2 × NH), 7.15 (dd, *J* = 1.2 and 8.1 Hz, 2H, 2 × H-6), 7.48 (d, *J* = 8.1 Hz, 2H, 2 × H-7), 7.52 (d, *J* = 1.2 Hz, 2H, 2 × H-4), 7.56 (d, *J* = 3.9 Hz, 1H, H-4'), 7.81-7.87 (m, 3H, H-3', H-2'' and H-6''), 8.31 (d, *J* = 8.1 Hz, 2H, H-3'' and H-5'') ppm. ^{13}C NMR ($CDCl_3$): δ = 28.30 ($C(\underline{C}H_3)_3$), 38.31 (β CH_2), 52.35 (CH_3), 54.57 (αC), 80.00 ($\underline{C}(CH_3)_3$), 110.25 (C7), 120.28 (C4), 126.38 (C4'), 126.30 (C6), 127.41 (C2'), 130.48 (C2'' and C6''), 130.93 (C3'), 132.84 (C5), 136.41 (C3'' and C5''), 138.49 (C1''), 144.00 (C5'), 142.44 (C3a), 149.80 (C7a), 151.78 (C4''), 155.11 (C=O Boc), 159.10 (C2), 172.03 (C=O ester) ppm. UV/Vis (ethanol, nm): λ_{max} (ε) = 363 (51,800). MS: *m/z* (ESI, %): 797 ([M+H]$^+$, 43), 740 (100). $C_{42}H_{44}N_4O_{10}S$: calcd. C 63.30, H 5.57, N 7.03; found C 63.23, H 5.43, N 7.12.

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CAPTIONS

Table 1. Yields, UV-visible absorption and emission data for *bis*-(oligo)thienylbenzoxazolyl-alanines **4a-d** in absolute ethanol and comparison with data for (oligo)thienylbenzoxazolyl-alanines **5a**[12b] and **5b-c**.[10c]

[a] in nm.

[b] in mol^{-1} L cm^{-1}.

[c] in cm^{-1}.

Figure 1. Normalized UV-visible absorption and emission spectra of compounds **4a-c** in absolute ethanol at T = 298 K (**4a**, λ_{exc} = 371 nm; **4b**, λ_{exc} = 388 nm; **4c**, λ_{exc} = 416 nm) (absorption, full line; emission, dotted line).

Figure 2. Structure of (oligo)thienylbenzoxazolyl-alanine derivatives **5a-c**.[12b, 10c]

Scheme 1. Synthesis of precursor diformylterthiophene **2c**.

Scheme 2. Synthesis of *N*- and *C*-terminus protected *bis*-(oligo)thienylbenzoxazolyl-alanine derivatives **4a-d**.

TABLES

Cpd.	π-bridge	Yield (%)	UV/Vis		Fluorescence			Cpd.	UV/Vis		Fluorescence		
			λ_{max}[a]	ε[b]	λ_{em}[a]	Stokes' shift[c]	Φ_F		λ_{max}[a]	ε[b]	λ_{em}[a]	Stokes' shift[c]	Φ_F
4a	tiophene	75	371	47,210	430	3698	0.89	**5a**[12b]	315	21,300	394	6365	0.80
4b	bithiophene	54	388	46,641	469	4451	0.59	**5b**[10c]	365	33,884	445	4925	0.46
4c	terthiophene	60	416	46,454	514	4583	0.14	**5c**[10c]	400	37,153	490	4592	0.10
4d	arylthiophene	76	363	51,800	440	4821	0.77	---	---	---	---	---	---

FIGURES

Figure 1.

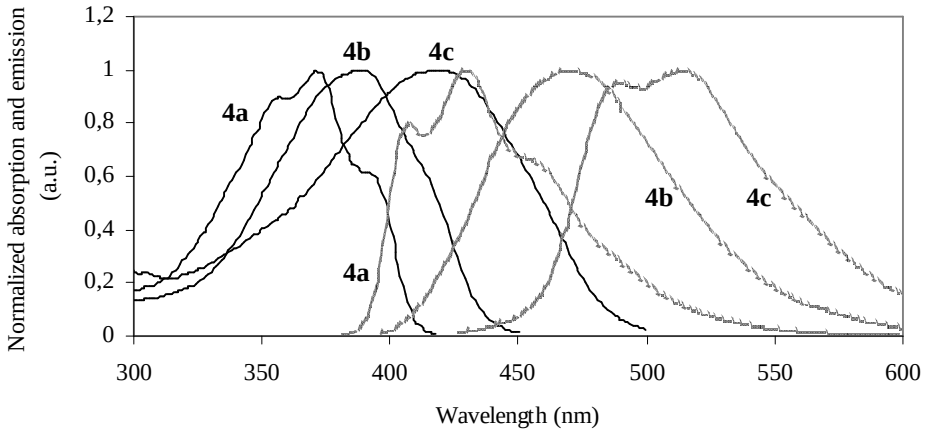
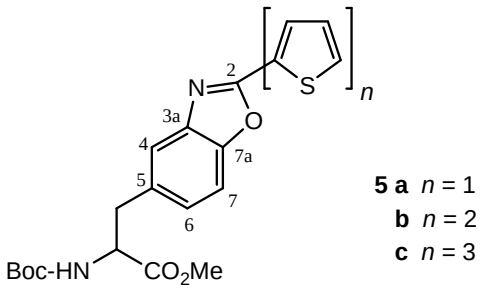


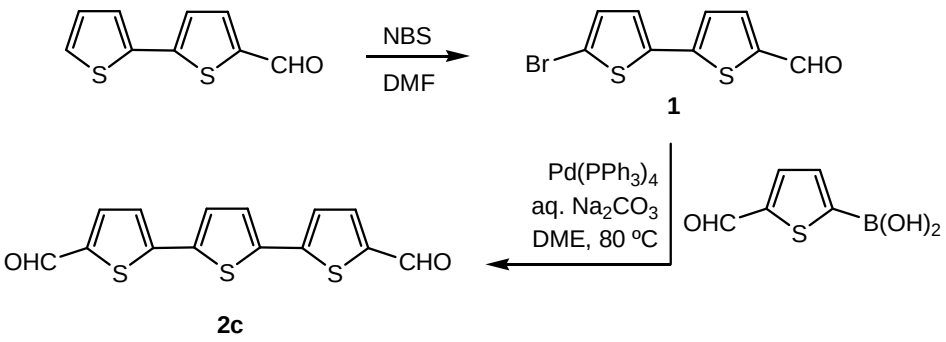
Figure 2.



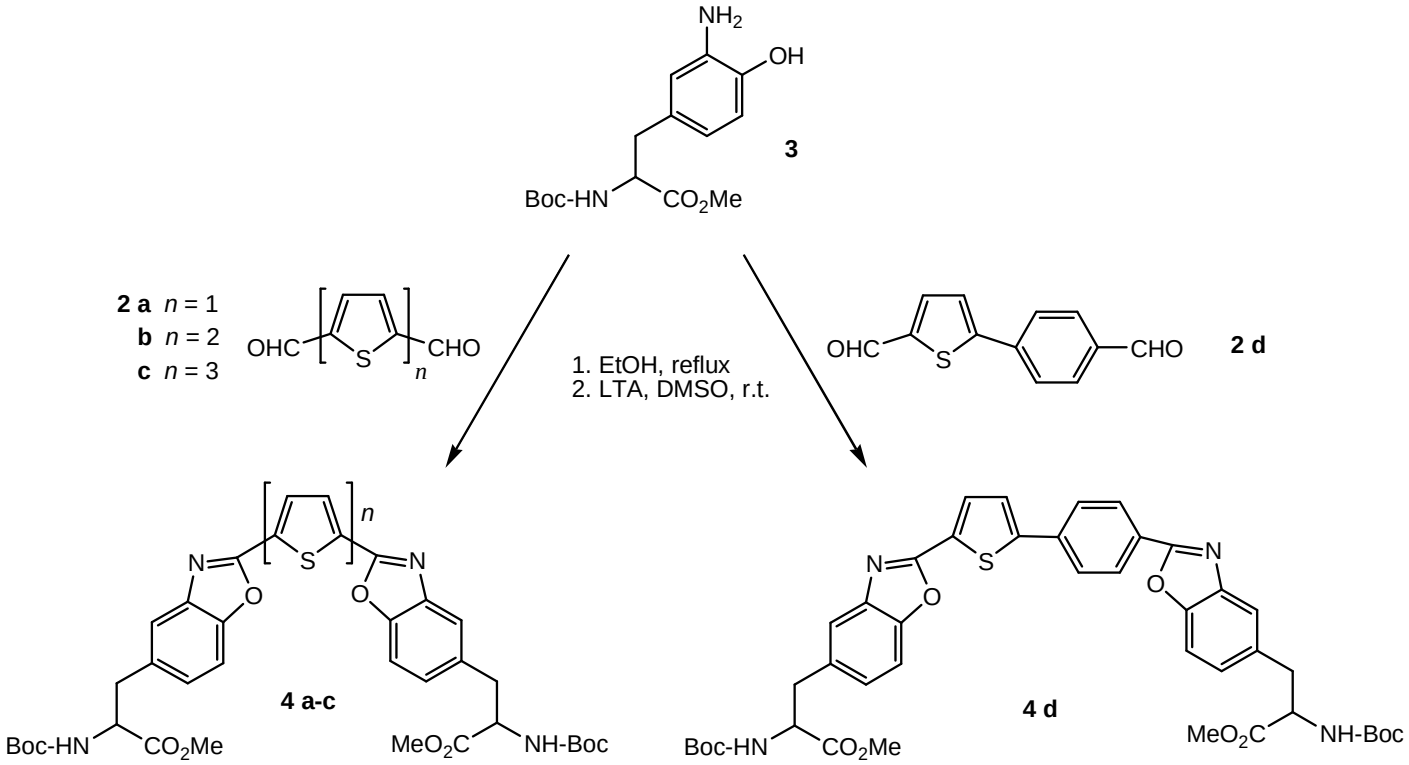
5 a *n* = 1
b *n* = 2
c *n* = 3

SCHEMES

Scheme 1.



Scheme 2.


NH2
OH
3
Boc-HN
CO2Me
2 a n = 1
b n = 2
c n = 3
OHC
S
CHO
n
1. EtOH, reflux
2. LTA, DMSO, r.t.
OHC
S
CHO
2 d
n
N
O
S
N
O
4 a-c
Boc-HN
CO2Me
MeO2C
NH-Boc
N
O
S
N
O
4 d
Boc-HN
CO2Me
MeO2C
NH-Boc